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Cellular localization of intrinsic factor in pancreas and stomach of the dog

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Summary. A cobalamin (vitamin B_{12})-binding protein has recently been identified in canine pancreatic juice which is biochemically, immunochemically and functionally similar to canine gastric intrinsic factor. However, the cellular sources of both this pancreatic intrinsic factor and gastric intrinsic factor in the dog are not known. Antisera raised against canine gastric intrinsic factor have been used to examine the distribution of intrinsic factors in the canine pancreas and stomach. Immunoreactivity was demonstrated in duct cells but not acinar or endocrine cells in the pancreas, and in fundic peptic and pyloric gastric pit cells in stomach. All immunostaining was abolished by preabsorption of the antisera with purified canine gastric and pancreatic intrinsic factors. A cellular source of pancreatic intrinsic factor has not been previously described, and the demonstration of intrinsic factor-like immunoreactivity in two cell types in the canine stomach contrasts with its localization in a single cell type in the gastric mucosa of other mammalian species. Furthermore, immunoreactivity in pancreatic duct cells was detected at much higher dilutions of antisera than those required for staining of peptic and gastric pit cells. This suggests a higher concentration of antigen, and supports previous evidence that the pancreas is a major source of intrinsic factor in the dog.

Key words: Intrinsic factor – Pancreas – Pancreatic intrinsic factor – Stomach – Immunohistochemistry – Dog

Intrinsic factor (IF) is a cobalamin (vitamin B_{12})-binding protein present in gastric juice which binds to specific receptors in the distal small intestine, and hence facilitates uptake of ingested cobalamin (Glass 1974; Marcoullis and Nicolas 1983). The other cobalamin-binding proteins in gastric juice, known as R binders, form unabsorbable complexes with cobalamin (Simons 1964; Grasbeck and Salonen 1976). Although IF is derived from gastric mucosa in all mammalian species studied (Glass 1974), immunohistochemical investigations have revealed species differences in the cellular sources of this glycoprotein (Jacob and Glass 1971; Levine et al. 1980, 1981, 1985).

The demonstration of impaired cobalamin absorption in human patients with exocrine pancreatic insufficiency (Cheney and Niemand 1932; Toskes et al. 1971) first implicated the pancreas in the normal handling of cobalamin, and introduced the possibility that the pancreas might be a source of IF. However, subsequent studies have suggested that pancreatic juice contributes to cobalamin absorption in man by degrading R binders, hence releasing cobalamin which can then bind to gastric IF (Allen et al. 1978; Marcoullis et al. 1980a; Toskes 1983; Marcoullis and Nicolas 1983).

In contrast to man, there is now considerable evidence that the pancreas plays a more fundamental role in the normal absorption of cobalamin in dogs. Firstly, despite the identification of IF in canine gastric juice (Marcoullis et al. 1980b) and of IF-cobalamin receptors in canine ileum (Marcoullis et al. 1980b; Seetharam et al. 1981; Levine et al. 1984), total gastrectomy does not appear to reduce cobalamin absorption (Abels and Muckerheide 1970; Abels et al. 1974, 1977). Secondly, low serum cobalamin concentrations have been reported in dogs with exocrine pancreatic insufficiency (Batt and Morgan 1982) and ligation of pancreatic ducts results in a severe malabsorption of cobalamin (Abels et al. 1974, 1977; Simpson et al. 1989) which can be reversed by oral administration of canine pancreatic juice (Simpson et al. 1989) or extracts of canine pancreas, even in dogs with total gastrectomy (Abels et al. 1974, 1977). Thirdly, a cobalamin-binding protein which is biochemically and immunochemically similar to canine gastric IF has recently been identified in canine pancreatic juice,

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and has been shown to promote ileal absorption of cobalamin by a physiological route (Batt et al. 1989; Batt and Horadagoda 1989). However, the cellular origins both of this pancreatic IF and also of gastric IF in the dog are not known.

Antisera raised against human and porcine IF have been used to localize IF in the gastric mucosa of various mammals (Levine et al. 1980, 1981, 1985), but canine IF appears to be immunochemically distinct from other mammalian IFs and shows weak or no cross-reactivity with such antisera (Yamaguchi et al. 1969; Abels and Muckerheide 1969, 1970). In the present study, antisera raised against IF purified from canine gastric mucosa (Batt et al. 1989) have been used to localize IF-immunoreactivity in canine pancreas and stomach. Preliminary reports of these findings have been published (Vaillant et al. 1986; Horadagoda et al. 1986).

Materials and methods

Gastric and pancreatic IFs were purified by affinity chromatography, as previously described (Horadagoda and Batt 1985; Batt et al. 1989). Successful purification of IFs and separation from R-binders was demonstrated by showing a lack of cobinamide inhibition of cobalamin-binding capacity, and a single band for both gastric and pancreatic IFs on SDS-polyacrylamide gel electrophoresis, corresponding to a molecular mass of approximately 53 kDa (Batt et al. 1989). The production of rabbit polyclonal antisera (R3 and R4) to purified canine gastric IF has been reported (Batt et al. 1989).

Tissues samples were taken from stomach (corpus and pyloric regions), pancreas (splenic and duodenal lobes, main duct) and salivary glands (parotid, mandibular) of normal dogs, from the pancreas of dogs with naturally occurring exocrine pancreatic insufficiency due to acinar atrophy (Hill et al. 1971), and from the stomach and pancreas of normal cats. Tissues were fixed in either Bouin's fluid or 4% formaldehyde solution and processed into wax. Samples of normal human stomach, processed in the same way, were available from a previous study (Taylor et al. 1981), and sections of formalin-fixed human pancreatic tumors containing some normal pancreas were kindly donated by Dr. G. Rothery, Department of Pathology, University of Liverpool Medical School.

The distribution of gastric IF-like immunoreactivity was examined by use of the peroxidase anti-peroxidase (PAP) method (Sternberger et al. 1970). Sections were incubated for 24 h with antisera R3 and R4 at serial dilutions from 1:100 to 1:64000, then for 1 h each with goat anti-rabbit IgG and rabbit PAP, both at 1:50 dilutions. The latter two antisera were purchased from ICN Biomedicals, Inc., UK. Sites of antibody binding were revealed by incubating sections for 10 min in substrate consisting of 0.025% diaminobenzidine and 0.015% hydrogen peroxide in 0.05 mol/l Tris buffer, pH 7.6. All reagents were purchased from Sigma, UK. In order to reduce background staining, endogenous peroxidase was blocked by incubating sections in aqueous 1% hydrogen peroxide for 20 min prior to staining, and by rinsing sections after each incubation in phosphate buffer (0.1 mol/l, pH 7.2) containing 2.5% sodium chloride (Grube 1980).

Crossreactivity of the two anti-gastric IF antisera with pancreatic IF was established by Western blotting (Batt et al. 1989). Specificity of the immunostaining was investigated by prior incubation of the primary antisera with purified gastric or pancreatic IFs (0.4 nmol IF/ml antiserum at 1:1000 dilution, for 48 h), and by replacing primary antisera with normal rabbit serum (1:50). The preparations of purified IFs used for these absorptions were those previously described (Batt et al. 1989).

Results

Immunohistochemical localizations of IF-like immunoreactivity in canine stomach, pancreas and salivary gland, were identical with R3 and R4 antisera, and all staining was abolished when the antisera were preabsorbed with either purified gastric IF or purified pancreatic IF. No staining was obtained when normal rabbit serum was substituted for the antisera.

In the pancreas, immunoreactivity was localized in the epithelium lining exocrine ducts: no staining was observed in pancreatic acinar or endocrine cells. Immunoreactivity was present in all epithelial cells in the main pancreatic duct and in interlobular ducts within the body of the pancreas, and occasionally also in the lumen (Fig. 1). In smaller intralobular ducts, there was an increasing proportion of non-immunoreactive cells as the ducts decreased in size (Fig. 2). A similar pattern of immunoreactivity was seen in the pancreas of dogs with exocrine pancreatic insufficiency in which there was a severe loss of exocrine cells but retention of the duct system and endocrine cells (Fig. 3). Immunoreactivity in the pancreas was detected by antisera R3 and R4 diluted to 1:64000.

In contrast, the optimal dilution of both antisera for demonstrating IF-like immunoreactivity in the stomach was 1:1000. Staining was observed in peptic cells in the fundic mucosa (Fig. 4a, b), and in columnar epithelial cells lining in the lower half of gastric pits in the pyloric mucosa (Fig. 5a, b). In the latter region, staining gradually decreased in epithelial cells closer to the surface of the mucosa, whereas there was an abrupt loss of immunoreactivity at the base of the gastric pits at junctions with pyloric glands (Fig. 5a). In the gastric pit cells, immunoreactivity was confined to the cytoplasm below the apical mucinogen granules (Fig. 5b).

In canine salivary glands, immunoreactivity was localized in only a few isolated epithelial cells in striated ducts in the mandibular gland (Fig. 6a, b), and none was observed in the parotid gland. Localizations were not obtained at dilutions of antisera higher than 1:1000.

No localizations were obtained in human and feline pancreas and stomach with antiserum R3. On the other hand, antiserum R4 did give weak localizations in human parietal cells only, and this staining was abolished by preabsorption with canine IF.

Discussion

Recent investigations have demonstrated that the pancreas is an important source of IF in the dog (Batt et al. 1989; Batt and Horadagoda 1989). The present study has pursued these findings and represents the first demonstration of the cellular source of IF in canine pancreas.

The localization of IF-like immunoreactivity in the pancreas and also in two cell types in the gastric mucosa in dogs contrasts with findings in other mammalian species where immunoreactivity appears to be confined to one cell type in either the fundic or pyloric regions of

Figs. 1–6. Canine tissues, immunoperoxidase localization of IF-like immunoreactivity and counterstaining with haematoxylin

Fig. 1. Canine gastric IF-like immunoreactivity is demonstrated in all lining cells and in the lumen (*arrow*) of an interlobular duct: exocrine acini (*Ex*) and islets (*I*) are unstained. ×120. *Bar*: 50μm

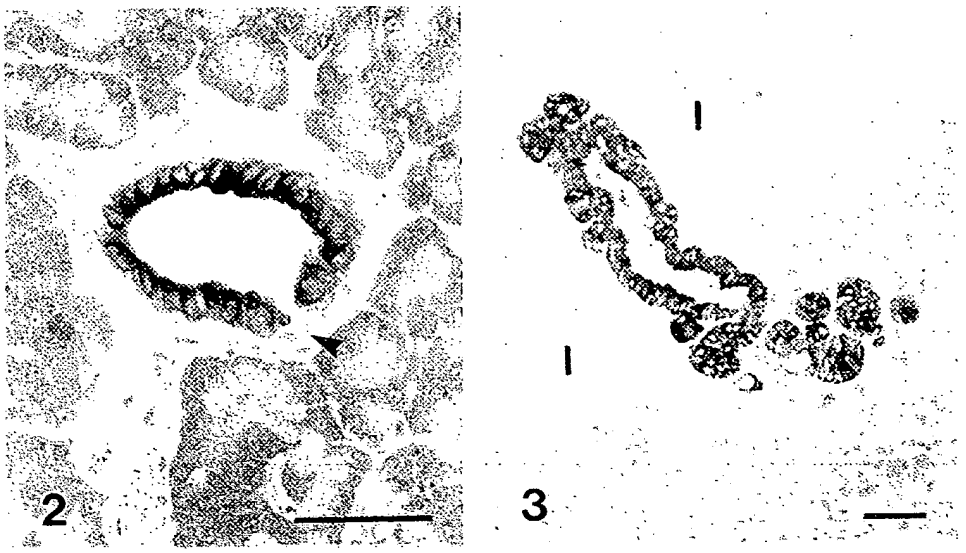

Fig. 2. IF-like immunoreactivity is present in the majority of cells lining an intralobular duct in dog pancreas except where a smaller duct branches off (*arrow*). ×360. *Bar*: 50 μm

Fig. 3. Pancreas from a dog with pancreatic acinar atrophy. Acinar tissue is severely reduced; IF-like immunoreactivity is present in ducts but not in surrounding islet tissue (*I*). ×190. *Bar*: 50 μm

the stomach (Jacob and Glass 1971; Levine et al. 1980, 1981, 1985). IF-immunoreactivity has been localized in parietal cells in man, guinea pigs and rabbits (Jacob and Glass 1971; Levine et al. 1980, 1981, 1985), but occurs in peptic cells in rats (Levine et al. 1981). The pig is quite different since IF-immunoreactivity has been demonstrated in cells lining the lower half of gastric pits in the pyloric antrum in this species (Levine et al. 1981). Thus, the localization of IF-like immunoreactivity in peptic cells and pyloric gastric pit cells in canine stomach indicates similarities both with the rat and with the pig, respectively. Antisera against canine gastric IF did not stain human and feline stomach or pancreas, apart from very weak staining of human parietal cells with antiserum R4, confirming earlier reports of immunochemical differences between IFs from dog and other mammalian species (Yamaguchi et al. 1969; Abels and Muckerheide 1969, 1970).

The possibility that a pancreatic IF might exist has been considered but has been refuted, at least in man (Allen et al. 1978; Marcoullis et al. 1980a; Marcoullis and Nicolas 1983; Toskes 1983; Carmel et al. 1983). However, pancreatic IF has been identified in the dog, and this protein has been shown to resemble canine gastric IF biochemically, immunochemically and functionally (Batt et al. 1989; Batt and Horadagoda 1989). The present study has extended these observations, and has demonstrated that pancreatic duct epithelium is the cellular source of this IF in canine pancreatic juice. The output of IF by the canine pancreas has been examined

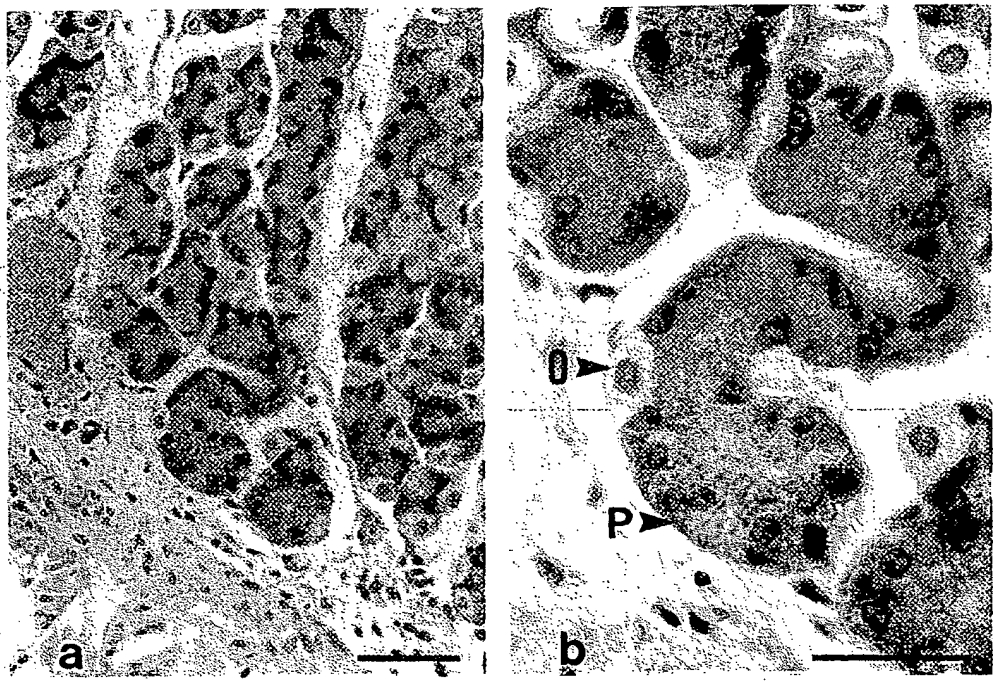

Fig. 4a, b. IF-like immunoreactivity in peptic cells (*P*), but not oxyntic cells (*O*) in canine fundic glands. a × 280, b × 720. *Bars*: a 50 μm; b 25 μm

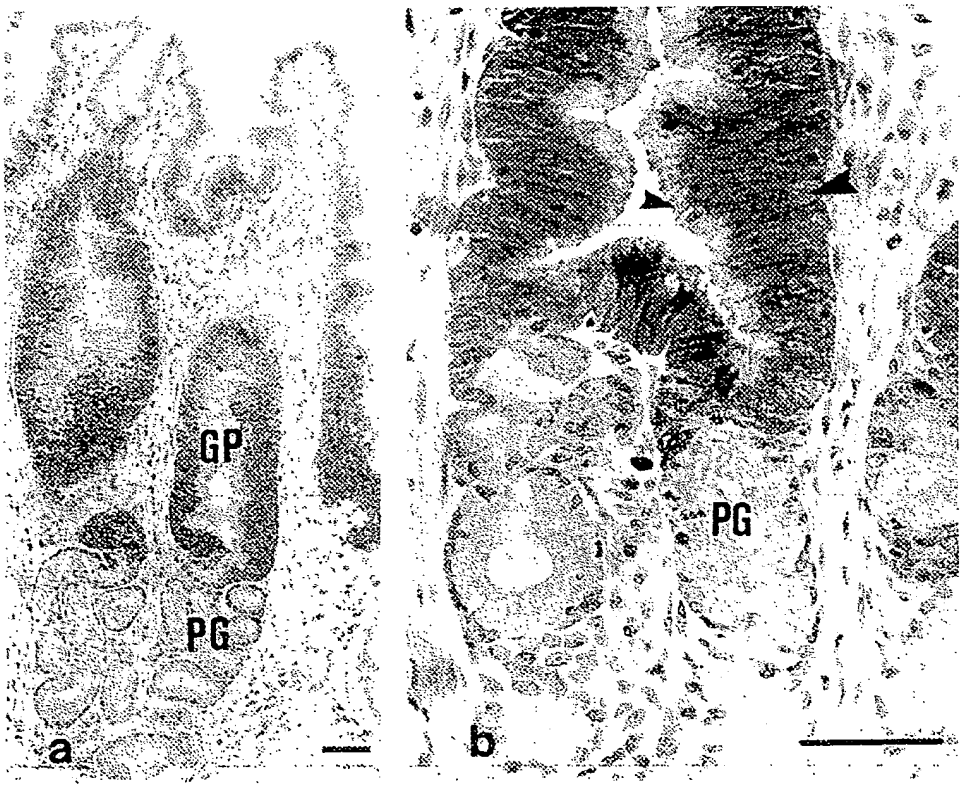

Fig. 5a, b. Immunoperoxidase demonstration of canine gastric IF-immunoreactivity in epithelial cells lining gastric pits in canine pyloric antral mucosa. Immunoreactivity increases towards the base of the gastric pits (*GP*), but is absent from the pyloric glands (*PG*). In individual gastric pit cells, staining occurs in the cytoplasm (*large arrow*) below the apical mucinogen granules (*small arrow*). a × 120, b × 360. *Bars*: 50 μm

following intravenous administration of secretin and cholecystokinin (CCK) (Batt et al. 1989). Stimulation of fluid output by secretin was associated with a relatively transient peak of IF, suggesting washout of material present in the lumen of ducts, whereas CCK resulted in a significantly greater output of IF which paralleled outputs of trypsin and protein, consistent with stimulation of IF secretion. Together with the identification of the cellular origin of pancreatic IF, these findings suggest that CCK can stimulate protein secretion by duct cells by a direct or indirect mechanism.

The demonstration of IF-like immunoreactivity in pancreatic ducts in dogs with pancreatic acinar atrophy suggests that these animals may retain the ability to produce pancreatic IF. Nevertheless, low serum concentrations of cobalamin (Batt and Morgan 1982) could reflect

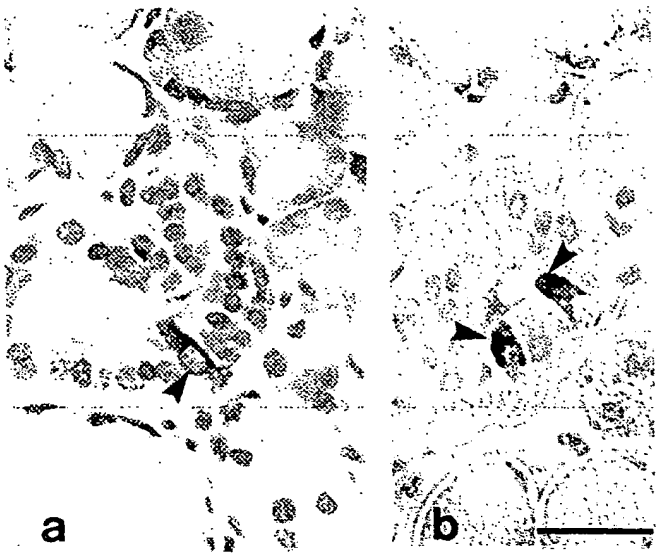

Fig. 6a, b. IF-like immunoreactivity occurs in isolated cells (*arrows*) in small striated ducts in mandibular gland (b phase contrast). ×550. *Bar*: 25 μm

impaired absorption of the vitamin perhaps due to reduced pancreatic fluid secretion (Sateri 1975) and hence defective output of pancreatic IF. However, alternative possibilities include reduced degradation of R-binders by pancreatic enzymes, or overgrowth of intestinal bacteria, and these have been discussed elsewhere (Simpson et al. 1989).

Kudo et al. (1987) have reported R binder-like immunoreactivity in ducts of human pancreas, a distribution which resembles that described here for IF in canine pancreas. The cellular source of canine R binders present in pancreatic juice (Batt et al. 1989) is unknown but it is possible that these may be derived from the same cell as canine pancreatic IF. In stomach and salivary gland, no other similarities were seen between our cellular localizations of IF-like immunoreactivity in dog and that of R binder-like immunoreactivity in man (Kudo et al. 1987).

Antigenicity was detected in the pancreas at much higher dilutions of antisera than those needed for staining in the stomach. The antisera were both raised against gastric IF and, although there are immunochemical similarities between canine pancreatic and gastric IFs (Batt et al. 1989), it is possible that the antisera may have a higher affinity for pancreatic than for gastric IF following tissue fixation. However, it is more likely that the difference in staining sensitivity reflects a much higher concentration of antigen in pancreatic duct cells than in peptic and gastric pit cells. This suggestion is supported by the five- to ten-fold higher output of IF in pancreatic secretion stimulated by secretin and CCK, respectively, compared with pentagastrin-stimulated IF output in gastric juice (Batt et al. 1989). While measurements of tissue concentrations of IF are needed to substantiate this suggestion, it is consistent with other evidence for a major role of the pancreas in the normal absorption of cobalamin by dogs (Batt et al. 1989; Batt and Horadagoda 1989; Simpson et al. 1989).

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